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Ochroconis terricola sp. nov. from China

Xin Zhang, Kun-Ying Wang, Peng-Peng Ren, Yu-Lan Jiang*

Department of Plant Pathology, Agriculture College, Guizhou University, Guiyang, 550025, China

*Correspondence to: *yljchsd@163.com*

Abstract—A new species, *Ochroconis terricola*, was isolated from soil in Guizhou Province, China. Morphology and phylogenetic analyses of the combined sequence data of nuclear ribosomal DNA genes (ITS, LSU, SSU) revealed the strain as different from other *Ochroconis* species.

Key words—*Dothideomycetes,* hyphomycetes, *Sympoventuriaceae,* taxonomy, *Venturiales*

Introduction

Ochroconis, typified by *O. constricta* (E.V. Abbott) de Hoog & Arx, was morphologically separated from *Scolecobasidium* E.V. Abbott (Abbott 1927) by de Hoog & von Arx (1974), for species with unbranched, ellipsoidal to cylindrical conidia, while *Scolecobasidium* was retained for species having T- or Y-shaped conidia. However, the taxonomic status of *Scolecobasidium* was questioned due to ambiguity of its type species, *S. terreum* E.V. Abbott, and species with lobed conidia similar to *S. terreum* in *Scolecobasidium* have been combined in *Ochroconis* on phylogenetic grounds (Samerpitak & al. 2014). Historically, *Ochroconis* was characterized by sympodial conidiogenesis and septate, mostly rough-walled conidia that are liberated rhexolytically (Samerpitak & al. 2015, Ellis 1971). A recent multigene (nSSU, nLSU, mtSSU, RPB2) phylogenetic analysis places *Ochroconis* in *Sympoventuriaceae* (*Venturiales*, *Dothideomycetes*) (Machouart & al. 2014). By combining molecular phylogeny, morphology, and ecology, Samerpitak & al. (2014) revised the taxonomy of the *Ochroconis* lineage, introducing

Verruconis Samerp. & al. as a new genus for a group of thermophilic species around *O. gallopava* (W.B. Cooke) de Hoog [≡ *Verruconis gallopava* (W.B. Cooke) Samerp. & de Hoog]. (*Ochroconis* species are mesophilic.) ITS and LSU nuclear ribosomal sequences as well as the conserved SSU gene are all suitable for identifying species in *Ochroconis* and *Verruconis*, but the ITS and LSU regions are recommended as the best DNA barcoding candidates, due to the high variability found even in conserved markers (Samerpitak & al. 2014, 2015). Presently, 31 species are accepted in *Ochroconis* (http://www.mycobank.org).

During our 2018 investigation of dematiaceous hyphomycete diversity, we isolated one *Ochroconis*-like strain from soil in Guizhou Province, China. Now we propose a novel species, *Ochroconis terricola,* based on morphological characters and phylogenetic analyses of ITS, LSU, and SSU nuclear ribosomal sequences.

Materials & methods

Fungal isolates and morphological studies

Soil samples were collected from Guizhou Province, China, in 2018 and transported to the laboratory in sterilized, zip lock polyethylene bags. All isolates are conserved in the Herbarium of Department of Plant Pathology, Guizhou University (HGUP). The taxon is described from cultures grown for 2 weeks at 25°C on potato dextrose agar (PDA). Conidia and conidiophores were placed in a drop of 60% lactic acid, examined, and photographed at 100× magnification using a Nikon 90i microscope.

DNA extraction, amplification, sequencing

Genomic DNA was extracted from colonies grown on PDA using the Fungal gDNA Kit GD2416 following the manufacturer's instructions. ITS, LSU and SSU genes were amplified via PCR using primers ITS4/ITS5 (White & al. 1990), LROR/LR5 (Rehner & Samuels 1994, Vilgalys & Hester 1990), and NS1/NS4 (White & al. 1990), respectively. The PCR products were purified and sequenced by Sangon Biotech. The new sequences were deposited in GenBank, and other sequences were obtained from GenBank (Table 1).

Phylogenetic analyses

Sequences were aligned using ClustalX 1.81 (Thompson & al. 1997) and edited manually using BioEdit (Hall 1999). The alignments were concatenated as a Fasta document using MEGA 6.0 (Tamura & al. 2011). Phylogenetic analyses of combined ITS, LSU, and SSU rDNA sequences were computed using maximum likelihood (ML) analysis and Bayesian Inference (BI). The ML tree was generated using RAxML-HPC2 on XSEDE v.8.2.8 (Stamatakis 2014) via the CIPRES Science Gateway platform (Miller & al. 2010) with 1000 bootstrap replicates. Bayesian analysis was performed via MrBayes v3.0b4 (Huelsenbeck & Ronquist 2001) using Markov Chain Monte

Table 1. Sequences used in phylogenetic analyses.

Species	Strain	GenBank accession no.		
		ITS	SSU	LSU
Mycosisymbrium cirrhosum	GUFCC 18012	KR259883	KR259885	KR259884
Ochroconis bacilliformis	CBS 100442	KP798632	KP798638	KP798635
O. constricta	CBS 202.27(T)	MH854929	KF156072	KF282652
	CBS 211.53	HQ667519	KF282671	KF282653
	NH 1234	—	—	LC187202
	FMR 3906	LM644509	—	LM644552
	NBRC 9375	DQ307327	AB564608	AB564619
O. cordanae	CBS 475.80(T)	KF156022	KF282672	KF282654
O. gamsii	CBS 239.78(T)	KF156019	KF156088	KF156150
O. icarus	CBS 536.69(T)	HQ667524	KF156084	KF156132
O. macrozamiae	CBS 101179	KF156020	KF156091	KF156151
	CBS 102491	KF156021	KF156092	KF156152
O. minima	CBS 423.64	—	KF282680	KF282666
	CBS 119792	KF156027	KF156086	KF156133
	CBS 510.71(T)	HQ667522	KF156087	KF156134
O. phaeophora	CBS 206.96	KP798631	KF282675	KF282660
O. ramosa	UTHSC 121082(T)	LM644524	LM644551	LM644567
O. robusta	CBS 112.97(T)	KP798633	KP798639	KP798636
O. sexualis	PPRI 12991(T)	KF156018	KF156089	KF156118
O. terricola	**HGUP1808(T)**	**MK377301**	**MK377071**	**MK377073**
Pleospora herbarum	CBS 191.86	KC584239	GU238232	GU238160
Scolecobasidium cateniphorum	CBS 769.83	KF156013	KF156044	KF156153
S. excentricum	CBS 469.95(T)	HQ667543	KF156096	KF156105
S. fusarioideum	CBS 210.95	—	KF156043	KF156154
S. fusiforme	CBS 586.82	KF156012	KF156101	KF156155
Scolecobasidium sp.	NH503	—	AB564606	AB564617
S. terreum	PO43	—	EU107356	EU107306
S. tricladiatum	PO51	—	EU107354	EU107286
S. tropicum	CBS 380.87	—	KF156042	KF156102
Venturia asperata	ATCC 34052	—	EF114736	EF114711
V. inaequalis	CBS 594.70	KF156040	GU296205	GU301879
V. populina	CBS 256.38	MH855959	GU296206	GU323212
V. pyrina	ATCC 38995	—	EF114739	EF114714
Veronaeopsis simplex	CBS 588.66(T)	KF156041	KF156095	KF156103
Verruconis calidifluminalis	CBS 125818(T)	MH875239	KF156046	KF156108
V. gallopava	CBS 437.64(T)	HQ667553	KF282674	KF282656
V. verruculosa	CBS 119775	KF156014	KF156055	KF156106

New sequence is set in **bold** font. Type strains are marked by T.

Carlo method. *Pleospora herbarum* (CBS 191.86) was used as an outgroup. The phylogenetic tree was viewed in Treeview v. 1.6.6 and the layout was completed in Adobe Illustrator CS5.

Taxonomy

Ochroconis terricola Xin Zhang & Y.L. Jiang, **sp. nov.** Fig. 1

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Differs from *Ochroconis macrozamiae* by its dark brown conidiophores and subhyaline to pale brown conidia, from *O. gamsii* by its flexuous conidiophores and bigger conidia, and from *O. sexualis* by its 1-septate and much smaller conidia.

Type: China, Guizhou Province, Leishan County, from forest soil, March 2018, X. Zhang (**Holotype**, HGUP1808; ex-type culture, HGUP1808; GenBank MK377301, MK377071, MK377073).

Etymology: referring to living on the soil.

Colonies on PDA effuse, flat, velvety, olivaceous, reverse dark brown, growing slowly, attaining 9 cm diam. at 25° after 4 weeks. Mycelium immersed and superficial, hyphae hyaline to pale brown, smooth. Conidiophores unbranched, 1–6 septa, flexuous, thick-walled, dark brown, 2–4 µm diam., with sympodially proliferating conidiogenous cells bearing one or more denticles in the apical region. Conidiogenous cells terminal, integrated, 1–3 µm long, polyblastic, cylindrical, subhyaline to medium brown. Conidia rhexolytic secession from conidiophores, solitary, subhyaline to pale brown, coarsely verrucose, 1-septate, sometimes slightly constricted at the septum, ellipsoidal to cylindrical or fusiform, apex mostly rounded, base narrowly truncated with minute marginal frills, 7–12 × 2–4 µm.

Phylogenetic analysis

The final alignment contained three genes for 36 isolates and 2568 (765 ITS + 827 LSU + 976 SSU) characters with *Pleospora herbarum* (CBS 191.86) as outgroup. The ML phylogenetic tree is shown (Fig. 2). Empirical base frequencies with 1,000 bootstrap inferences are 0.249211 (pi A), 0.221282 (pi C), 0.295026 (pi G), and 0.234480 (pi T). Bayesian analysis generated a similar phylogenetic tree (one million generations; average standard deviation of split frequencies = 0.009724). The *Ochroconis terricola* (HGUP 1808) sequences cluster together in a clade with *O. macrozamiae* Crous & R.G. Shivas, *O. gamsii* de Hoog, and *O. sexualis* Samerp. & al. (Fig. 2) and form a single branch sister to the other three species with high bootstrap support (ML/BI = 84/1).

Discussion

In this study, one *Ochroconis* strain (HGUP1808) isolated from soil was identified based on morphological characters and molecular data.

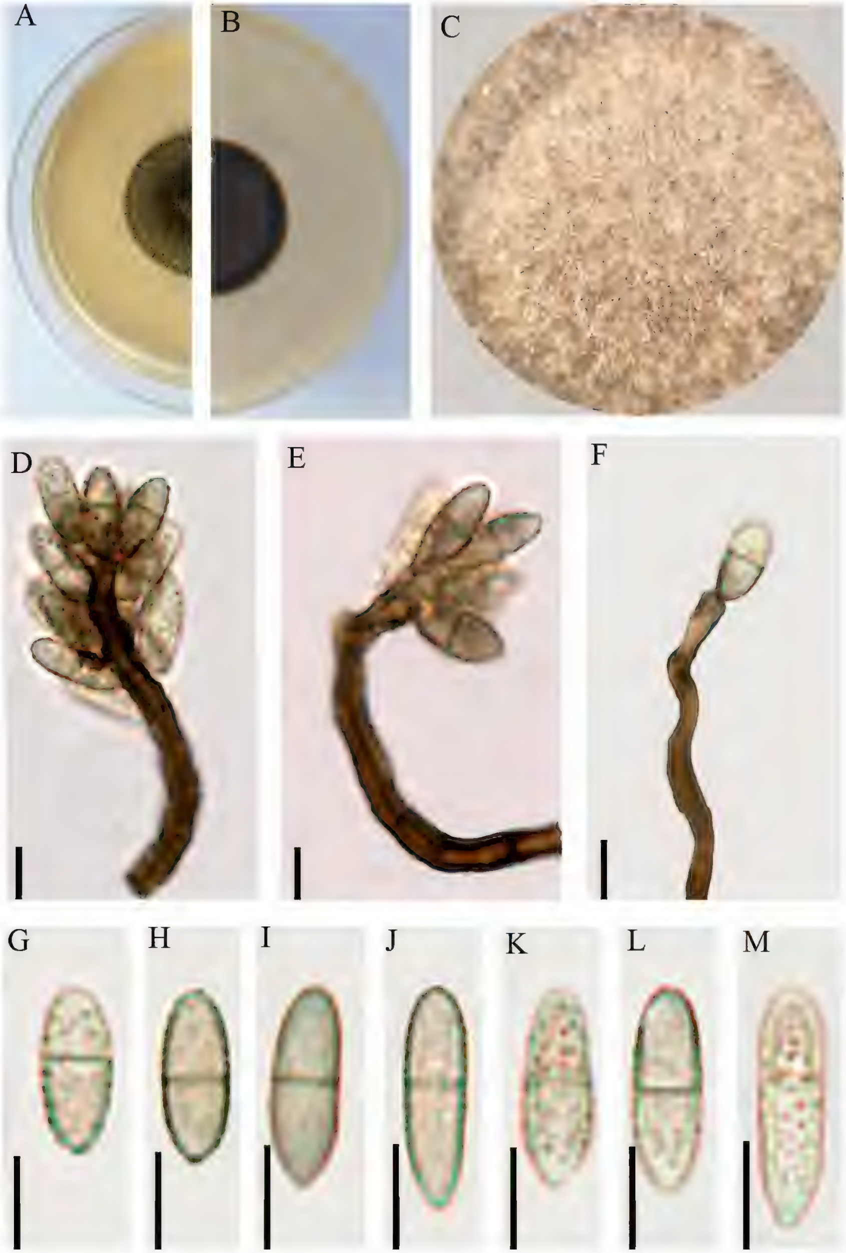

Fig. 1. *Ochroconis terricola* (holotype, HGUP1808). A, B: Colony on PDA at 14 days; C: Mycelia on the colony; D–F: Conidiophores and conidia; G–M: Conidia. Scale bars = 5 µm.

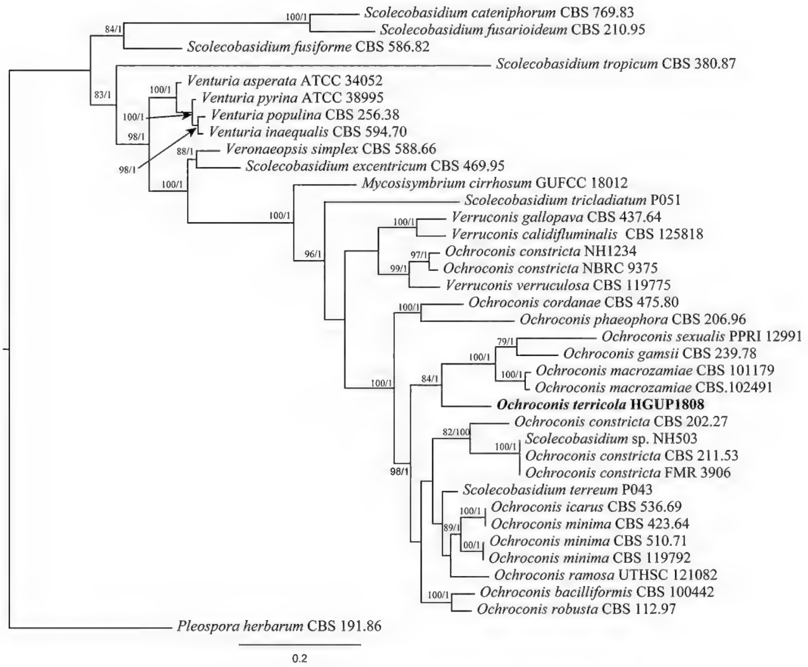
100/1
Scolecobasidium cateniphorum CBS 769.83
84/1
Scolecobasidium fusarioideum CBS 210.95
Scolecobasidium fusiforme CBS 586.82
Scolecobasidium tropicum CBS 380.87
83/1
100/1
Venturia asperata ATCC 34052
Venturia pyrina ATCC 38995
100/1
Venturia populina CBS 256.38
98/1
Venturia inaequalis CBS 594.70
88/1
Veronaeopsis simplex CBS 588.66
98/1
Scolecobasidium excentricum CBS 469.95
100/1
Mycosisymbrium cirrhosum GUFCC 18012
Scolecobasidium tricladiatum P051
100/1
100/1
Verruconis gallopava CBS 437.64
Verruconis calidifluminalis CBS 125818
96/1
97/1
Ochroconis constricta NH1234
99/1
Ochroconis constricta NBRC 9375
Verruconis verruculosa CBS 119775
100/1
Ochroconis cordanae CBS 475.80
Ochroconis phaeophora CBS 206.96
79/1
Ochroconis sexualis PPRI 12991
100/1
Ochroconis gamsii CBS 239.78
100/1
Ochroconis macrozamiae CBS 101179
100/1
84/1
Ochroconis macrozamiae CBS.102491
Ochroconis terricola HGUP1808
Ochroconis constricta CBS 202.27
82/100
Scolecobasidium sp. NH503
100/1
Ochroconis constricta CBS 211.53
98/1
Ochroconis constricta FMR 3906
Scolecobasidium terreum P043
100/1
Ochroconis icarus CBS 536.69
89/1
Ochroconis minima CBS 423.64
00/1
Ochroconis minima CBS 510.71
Ochroconis minima CBS 119792
Ochroconis ramosa UTHSC 121082
100/1
Ochroconis bacilliformis CBS 100442
Ochroconis robusta CBS 112.97
Pleospora herbarum CBS 191.86
0.2

Fig. 2. Maximum Likelihood (ML) tree based on combined LSU, ITS, and SSU data of *Ochroconis* species and other related genera species, with *Pleospora herbarum* (CBS 191.86) as outgroup. Branch support is shown as: Maximum Likelihood bootstrap support values >75% / Bayesian posterior probabilities >0.95. The new species is in bold.

SSU, ITS, and LSU multi-gene analyses support phylogenetic separation of *O. terricola* from known *Ochroconis* species within a single clade in the ML tree. Although *Ochroconis terricola* is phylogenetically close to *O. macrozamiae, O. gamsii*, and *O. sexualis* in the phylogram (Fig. 2), there are obvious morphological differences separating *O. terricola* and the three species. *Ochroconis macrozamiae* is distinguished by its red-brown conidiophores and conidia that constrict at the middle septum (Crous & al. 2014); *O. gamsii* differs by its erect conidiophores and curved or unilaterally flattened, smaller conidia (6–9 × 2.4–2.8 µm; de Hoog 1985); and *O. sexualis* differs by its 1–3-septate, larger conidia (15–22 × 3.5–5.0 µm; Samerpitak & al. 2014). The combined morphological and phylogenetic analysis supports *O. terricola* as a new taxon.

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Literature cited

Abbott EV. 1927. *Scolecobasidium*, a new genus of soil fungi. Mycologia 19(1): 29–31. https://doi.org/10.2307/3753662

Crous PW, Shivas RG, Quaedvlieg W, van der Bank M, Zhang Y, Summerell BA & al. 2014. Fungal Planet Description Sheets: 214–280. Persoonia 32: 184–306. https://doi.org/10.3767/003158514X682395

de Hoog GS, von Arx JA. 1974 ["1973"]. Revision of *Scolecobasidium* and *Pleurophragmium*. Kavaka 1: 55–60.

de Hoog GS. 1985. Taxonomy of the *Dactylaria* complex, IV. *Dactylaria*, *Neta*, *Subulispora* and *Scolecobasidium*. Studies in Mycology 26: 1–60.

Ellis MB. 1971. Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. 608 p.

Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/ 98/ NT. Nucleic Acids Symposium Series 41: 95–98.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755. https://doi.org/10.1093/bioinformatics/17.8.754

Machouart M, Samerpitak K, de Hoog GS, Gueidan C. 2014. A multigene phylogeny reveals that *Ochroconis* belongs to the family *Sympoventuriaceae* (*Venturiales*, *Dothideomycetes*). Fungal Diversity 65: 77–88. https://doi.org/10.1007/s13225-013-0252-7

Miller MA, Pfeiffer W, Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, p. 1–8. https://doi.org/10.1109/GCE.2010.5676129

Rehner SA, Samuels GJ. 1994. Taxonomy and phylogeny of *Gliocladium* analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625–634. https://doi.org/10.1016/S0953-7562(09)80409-7

Samerpitak K, Van der Linde E, Choi HJ, Gerrits van den Ende AHG, Machouart M, Gueidan C, Hoog GS de. 2014. Taxonomy of *Ochroconis*, genus including opportunistic pathogens on humans and animals. Fungal Diversity 65: 89–126. https://doi.org/10.1007/s13225-013-0253-6

Samerpitak K, Duarte APM, Attili-Angelis D, Pagnocca FC, Heinrichs G, Rijs AJMM, Alfjorden A, Gerrits van den Ende AHG, MenKen SBJ, de Hoog GS. 2015. A new species of the oligotrophic genus *Ochroconis* (*Sympoventuriaceae*). Mycological Progress 14, Article number 6: 1–10. https://doi.org/10.1007/s11557-015-1023-5

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28(10): 2731–2739. https://doi.org/10.1093/molbev/msr121

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882. https://doi.org/10.1093/nar/25.24.4876

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172: 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

White TJ, Bruns T, Lee SB, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis MA & al. (eds). PCR protocols, a guide to methods and applications. San Diego: Academic Press. https://doi.org/10.1016/B978-0-12-372180-8.50042-1